Pancreatic stone protein predicts sepsis in severely burned patients irrespective of trauma severity

Klein, Holger Jan ; Niggemann, Pia ; Buehler, Philipp Karl ; Lehner, Fabienne ; Schweizer, Riccardo ; Rittirsch, Daniel ; Fuchs, Nina ; Waldner, Matthias ; Steiger, Peter ; Giovanoli, Pietro ; Reding, Theresia ; Graf, Rolf ; Plock, Jan Alexander

Abstract: OBJECTIVE: The burn victim's inherent state of hyperinflammation frequently camouflages septic events delaying the initiation of targeted intensive care therapy. Accurate biomarkers are urgently needed to support sepsis detection before patients' clinical deterioration. SUMMARY OF BACKGROUND DATA: Evidence on the usefulness of pancreatic stone protein (PSP) as a powerful diagnostic and prognostic marker in critically ill patients has recently accumulated. METHODS: Analysis of biomarker kinetics (PSP, routine markers) was performed on 90 patients admitted to the Zurich Burn Center between May 2015 and October 2018 with burns 15% total body surface area with regard to infection and sepsis (Sepsis-3) over a 14-day time course. RESULTS: PSP differentiated between sepsis, infection and sterile inflammation from day 3 onward with an area under the curve of up to 0.89 ($P < 0.001$), therefore, competing with procalcitonin (area under the curve = 0.86, $P < 0.001$). Compared to routine inflammatory biomarkers, only PSP demonstrated a significant interaction between time and presence of sepsis - signifying a steeper increase in PSP levels in septic patients as opposed to those exhibiting a nonseptic course (interaction $P < 0.001$). Event-related analysis demonstrated tripled PSP serum levels within 72 hours and doubled levels within 48 hours before a clinically apparent sepsis. CONCLUSION: PSP is able to differentiate between septic and nonseptic patients during acute burn care. Its steep rise up to 72 hours before clinically overt deterioration has the potential for physicians to timely initiate treatment with reduced mortality and costs.

DOI: https://doi.org/10.1097/sla.0000000000003784

Pancreatic Stone Protein Predicts Sepsis in Severely Burned Patients Irrespective of Trauma Severity

A Monocentric Observational Study

Holger Jan Klein, MD,†✉ Pia Niggemann, MD,* Philipp Karl Buehler, MD,‡ Fabienne Lehner, MD,* Riccardo Schweizer, MD,*† Daniel Rittirsch, MD,* Nina Fuchs, MD,*† Matthias Waldner, MD,*† Peter Steiger, MD,‡ Pietro Giovanoli, MD,* Theresia Reding,§ Rolf Graf, PhD,§ and Jan Alexander Plock, MD*†*

Objective: The burn victim's inherent state of hyperinflammation frequently camouflages septic events delaying the initiation of targeted intensive care therapy. Accurate biomarkers are urgently needed to support sepsis detection before patients' clinical deterioration.

Summary of Background Data: Evidence on the usefulness of pancreatic stone protein (PSP) as a powerful diagnostic and prognostic marker in critically ill patients has recently accumulated.

Methods: Analysis of biomarker kinetics (PSP, routine markers) was performed on 90 patients admitted to the Zurich Burn Center between May 2015 and October 2018 with burns ≥15% total body surface area with regard to infection and sepsis (Sepsis-3) over a 14-day time course.

Results: PSP differentiated between sepsis, infection and sterile inflammation from day 3 onward with an area under the curve of up to 0.89 ($P < 0.001$), therefore, competing with procalcitonin (area under the curve = 0.86, $P < 0.001$). Compared to routine inflammatory biomarkers, only PSP demonstrated a significant interaction between time and presence of sepsis – signifying a steeper increase in PSP levels in septic patients as opposed to those exhibiting a nonseptic course (interaction $P < 0.001$). Event-related analysis demonstrated tripled PSP serum levels within 72 hours and doubled levels within 48 hours before a clinically apparent sepsis.

Conclusion: PSP is able to differentiate between septic and nonseptic patients during acute burn care. Its steep rise up to 72 hours before clinically overt deterioration has the potential for physicians to timely initiate treatment with reduced mortality and costs.

Keywords: biomarker analysis, kinetics of biomarkers, pancreatic stone protein, PSP, sepsis biomarker

(*Ann Surg* 2020;xx:xxx–xxx)

From the *Department of Plastic Surgery and Hand Surgery, Burn Center, University Hospital Zurich, Zurich, Switzerland; †Regenerative and Reconstructive Plastic Surgery Research Laboratory, University of Zurich, Zurich, Switzerland; ‡Institute of Intensive Care Medicine, University Hospital Zurich, Zurich, Switzerland; and §Pancreas Research Laboratory, Department of Visceral Surgery & Transplantation, University Hospital Zurich, Zurich, Switzerland.

✉holger.klein@usz.ch.

Reprints will not be available from the authors.

Rolf Graf is the inventor of an assay covered by patent No: EP 2185937 B2 ''METHOD FOR ASSAYING SEPSIS IN HUMANS,'' which is owned by the University of Zurich (Zurich, Switzerland). This does not alter the authors' adherence to all policies of Annals of Surgery on sharing data and materials.

This study was supported by a grant from the Gottfried and Julia Bangerter-Rhyner Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The authors report no conflicts of interest.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.annalsofsurgery.com).

ISSN: 0003-4932/16/XXXX-0001

DOI: 10.1097/SLA.0000000000003784

Although the overall survival of extensive burns has increased over time due to sophisticated burn wound care, radical infection control measures, altered fluid resuscitation protocols and enhanced nutritional support, mortality remains substantial with up to 50% and is, therefore, higher than that associated with heart failure or most cancer rates.[1–7] The incidence of septic courses secondary to thermal injuries varies in literature between 3% and 30% for cases with burns covering over 15% total body surface area (TBSA).[4,8]

A major limiting factor for the poor clinical outcome of septic patients – even under best possible care – is the lack of reliable diagnostic tools with the prompt recognition of critical events.[9] This is even more challenging in burns, as thermal injuries lead to a state of hyperinflammation, capillary leakage and hypermetabolism triggering dysfunctional catabolic circuits in nearly every organ system.[4,10,11] Clinical signs expressing the enormous stress caused by the thermal injury itself such as fever, hypothermia, tachycardia, or hyperventilation frequently mask infectious events and, therefore, make the delineation between sterile inflammatory processes and infectious/septic progressions significantly challenging. Consequently, physicians depend on blood biomarkers to help uncover infectious events at an incipient stage of the disease. Though, established inflammatory biomarkers such as white blood cells (WBCs), C-reactive protein (CRP), procalcitonin (PCT), or interleukin-6 often fail in that respect – especially in burn patients – for different reasons.[12–15] Likewise, novel promising biomarkers derived from the omics or inflammasome families are currently being investigated, but for now lack broader evidence, availability, and affordability.[16–18]

Evidence on the usefulness of the pancreatic stone protein (PSP) as an accurate diagnostic and prognostic marker in critically ill patients has recently been increasing.[19] Originally described as a protein constitutively secreted by pancreatic acinar cells to inhibit growth and nucleation of calcium carbonate crystals, insights from more recent studies suggested PSP as an acute phase protein activating neutrophil granulocytes in the early phase of infection leading to an appreciation of a much broader functional scope. Serum PSP levels were found to be highly predictive for infectious and septic courses of neonates, trauma patients, cardiac surgery patients, emergency patients as well in a broad range of intensive care unit (ICU) patients with areas under the curve (AUC) of up to 0.91, thus being able to compete with well-established biomarkers like PCT.[20–25] Reding and colleagues have further emphasized the role of the pancreas as an acute phase organ by sensing remote tissue damage, therefore, triggering the release of PSP, particularly when associated with septic complications.[26,27]

Especially in burn patients, where early decision making with initiation of therapy is key to survival, inflammatory biomarkers are urgently needed aiming at the preclinical detection of deterioration to sepsis or septic shock. Serum levels of circulating PSP and their

predictive usefulness for infection and sepsis have not yet been studied in this critically ill population so far. In this study, we analyzed the first 14 days of serum PSP in a cohort of 90 severely burned patients (≥15% TBSA) to determine its discriminatory accuracy to delineate sterile systemic inflammation from infectious/septic courses as compared to currently available inflammatory biomarkers (WBCs, CRP, PCT). We particularly focused on the temporal change of PSP serum levels before the clinically overt septic event.

METHODS

Type of Study

Observational study

Ethics Approval

Approval was obtained from the Ethics committee of the University of Zurich, Switzerland on April 20th 2015 (KEK-ZH-No: 2014-0631).

Sample Size

We focused on the interaction between time (14 time points) and status of sepsis (ie, 2 groups) as the most relevant hypothesis, because bedside clinicians are interested in the change over time of a biomarker to detect a patient's deterioration. Previous findings on PSP in infected patients performed by our group revealed an effect size Cohen $f = 0.2$ (0.10, 0.25, and 0.40 represent small, medium, and large effect sizes, respectively).[21] Due to the burn patients inherent baseline inflammation potentially interfering with biomarker levels in infected/septic patients, we chose an even lower, conservative effect size of about 0.1–0.15 for the present study. Based on the these pre-study considerations, a priori sample size estimation was performed using GPOWER 3.1 resulting in 82 patients required (given $\alpha = 0.05$, power = 0.9, effect size = 0.1, number of measurements = 14, correlation among measurements = 0.5).[28]

Participants

Between May 2015 and October 2018, patients with burns ≥15% TBSA admitted to our burn center were asked for participation. Affected TBSA was determined using Lund Browder charts. A minimum TBSA of 15% was set for inclusion based on evidence of systemic stress, impaired immunity, and massive fluid shifts.[8,29] Exclusion criteria were age <18 years, current infection at admission, immunosuppressive medication, and burn injuries older than 6 hours. All patients received comprehensive oral and written information on the present study and had to sign the informed consent for enrollment. Close relatives and authorized representatives were asked for informed consent by proxy, if the patient was unable to consent due to the extent of the injury.

Measurement of Serum/Plasma Biomarker Concentration

Blood samples were drawn daily at 6 AM, starting at admission to our burn center, for measurement of conventional inflammatory biomarkers (CRP, WBCs, and PCT) and PSP. Leukocyte counts, CRP and PCT levels were directly measured by routine testing at the Institute of Clinical Chemistry, University Hospital Zurich. For subsequent analysis, serum samples were stored at −80°C. The concentration of PSP/REG Iα was measured with an isoform specific enzyme-linked immunosorbent assay, which was established in our laboratory.[30,31] Antibodies (affinity-purified IgG) made in guinea pig anti-PSP/REG Iα were diluted 1:500 in Tris-buffered saline (TBS: 10 mM Tris; 0.9% NaCl) and coated on 96-well Maxisorp Nunc plates at room temperature over night or at 4°C if incubation lasted longer than 1 night. Nonadherent antibodies were removed with 3 washing steps. The wash buffer consisted of TBS and 0.05% Tween. Bovine serum albumin (BSA, tested for low level PSP/REG Iα content) 1% in TBS was used to block the plates for at least 1 hour at room temperature. Serum samples collected from patients were pre-diluted in 1% BSA/TBS and loaded on the plate in duplicates. The standard curve was generated by a dilution series ranging from 4 ng/mL and 0.1 ng/mL, prepared with recombinant PSP. In addition, a blank was added. After 1 hour of incubation and 3 washing steps a secondary antibody, rabbit anti-PSP/REG Iα, 1:500 diluted in 1% BSA/TBS was incubated in the plate. Subsequently, a biotinylated phosphatase antibody 1:6000 diluted in 1% BSA TBS was added for 1 hour. After another washing step, the phosphatase substrate (SIGMA Aldrich, Buchs, Switzerland) was added, which was previously dissolved in alkaline phosphatase buffer according to instructions. PSP was detected photometrically at 405 nm.

Patient-related Data

Besides demographic data [age, sex, body mass index (BMI), comorbidities] and trauma-related data (TBSA, mechanism of injury, inhalation injury), clinical parameters were extracted from the patients' chart retrospectively and collected in the case report form for 14 consecutive days after admission. The latter included: blood count, electrolytes, inflammatory markers (PCT, CRP), liver, kidney, and pancreas function. The abbreviated burn severity index (ABSI) score was used as prognostic stratification system including age, sex, TBSA, third degree burns, and inhalation injury.[32] Additionally, vital signs and data on circulatory support, ventilation, and mental status were collected to closely study pathophysiological changes with regard to infection and sepsis. Clinical parameters at the timepoint of blood sampling were chosen for analysis (usually at 6 AM daily). All treating physicians were blinded to PSP results whereas they were aware of WBCs, CRP, and PCT values. We used the Centers for Disease Control and Prevention-definition for hospital acquired infections and the Third International Consensus Definition for Sepsis and Septic Shock (Sepsis-3).[33,34] The corresponding time point of any infection or sepsis was determined by the date of sampling, which subsequently turned out positive.

Statistical Analysis

Discrete values are expressed as counts with percentages, whereas continuous variables are presented as mean ± standard deviation or median with interquartile range (IQR) as appropriate. Baseline characteristics were compared between groups using Chi-square test for counts and univariate analysis of variance for continuous data. Pearson product-moment correlation was performed to identify associations between levels of biomarkers at admission and continuous baseline characteristics. Biomarker time courses were compared between groups using a linear mixed effects regression model with random intercepts. Linear mixed models have proven superior to traditional repeated-measures analysis of variance as they properly take into account interindividual differences in complex longitudinal data.[35] Univariate binary logistic regression analysis was used to test PSP, PCT, CRP, and WBC as predictors for the clinical outcome. Receiver operating characteristics analysis was employed at each day to evaluate the diagnostic/prognostic performance of the biomarkers tested with regard to infection and sepsis. Values of the AUC are reported with corresponding 95%-confidence interval. Cut-off points for PSP levels were selected giving equal weight to sensitivity and specificity. All tests were 2 tailed; $P < 0.05$ was considered significant. Data were analyzed using SPSS (Statistical Package for Social Sciences, Version 24 for Macintosh; SPSS Inc., Chicago, IL) and GraphPad Prism version 6.00 for Macintosh (GraphPad Software, La Jolla CA).

RESULTS

Baseline Characteristics of Study Population

Baseline characteristics of the study population in total and according to the main outcome (no infection vs infection vs sepsis vs septic shock) are given in Table 1. Ninety severely burned patients (18 female) with TBSA ≥ 15% were included. We pooled the septic and septic shock group for further analyses due to the low number of patients with septic shock (n = 7). Thirteen patients (14%) exhibited a local infection, whereas 46 patients (51%) developed septic complications within the first 14 days. Mean age was 48.5 ± 18.8 years and did not differ between the 3 groups ($P = 0.595$). Total median TBSA was 31.5% (IQR 21%) and differed significantly in the subgroup analysis [no infection: 29% (IQR 24%), local infection: 25% (IQR 18%), sepsis: 35% (IQR 26%); $P = 0.048$]. Fourteen patients (16%) died. Septic/infected patients had a comprehensibly longer stay in the ICU and longer total length of stay in the hospital than patients with an uneventful course ($P < 0.001$). We focused on the first infectious event that occurred within the first 14 days observing pneumonia in 34 patients (58%), followed by wound infections in 12 patients (20%), 6 patients with central line infection (10%), bacteremia in 4 patients (7%), and 3 patients (5%) with urinary tract infection (Table 1).

Association Between Inflammatory Markers and Baseline Characteristics

Age and BMI did not correlate with PSP, WBCs, and PCT at baseline ($P > 0.100$), whereas CRP at baseline showed a mild association with age ($r = 0.26$, $P = 0.021$) and BMI ($r = .28$, $P = 0.019$). TBSA did not correlate with levels of PSP, PCT, and CRP at baseline, but was moderately associated with WBCs at baseline ($r = .55$, $P < 0.001$). A linear mixed effect regression model investigating the influence of TBSA over 14 days showed a significant interaction between time and TBSA for all biomarkers, which is why all subsequent analyses were adjusted for TBSA (interaction for all biomarkers $P < 0.001$). In an equal model, age had a significant effect on the time course of PSP (interaction $P = 0.014$). However, when adjusting the latter model for TBSA, age lost its significant effect (interaction $P = 0.390$). All biomarker levels were unaffected by sex and the presence of diabetes mellitus ($P > 0.100$). The 14-day time course of all biomarkers according to the median split for age (52 years) and TBSA (31.5%) is given as supplemental material (Supplemental 1, http://links.lww.com/SLA/B969 and 2, http://links.lww.com/SLA/B969). Additionally, PSP levels of 32 patients drawn at a later time point (at least 3 months after discharge from hospital) did not differ from PSP at admission in paired samples *t*-test ($P = 0.094$) underlining the validity of the baseline values as not being influenced by trauma severity.

Time Course of Inflammatory Biomarkers as Related to Infection and Sepsis

To delineate sterile inflammation from infection and sepsis, we focused on the first 10 days after trauma encompassing >90% of the outcome events and adjusted the model for TBSA. Using linear mixed effect regression analysis, PSP was able to differentiate between infection, sepsis and sterile inflammation from day 3 ongoing, whereas PCT distinguished between the 3 groups at every time point. CRP was able to differentiate between noninfection, infection,

TABLE 1. Baseline Characteristics in Total and According to the Main Outcome No Infection Versus Infection Versus Sepsis Versus Septic Shock. For Group Comparison, Sepsis and Septic Shock Patients Were Clustered. Counts Were Compared Row-wise Using Chi-square Test, Continuous Variables Were Tested With Univariate ANOVA After Log10-Transformation Where Appropriate

	Total	No Infection	Local Infection	Sepsis	Septic Shock	*P*
Number of patients	90	31 (34.4%)	13 (14.4%)	39 (43.3%)	7 (7.8%)	—
Sex (n, %)						
Female	18 (20%)	6 (33.3%)	3 (16.7%)	7 (38.9%)	2 (11.2%)	0.956
Male	72 (80%)	25 (34.7%)	10 (13.9%)	32 (44.4%)	5 (6.9%)	
Age (yr; mean ± SD)	48.5 ± 18.8	51.3 ± 20.4	46.6 ± 17.6	47.6 ± 18.1	44.1 ± 20.6	0.595
TBSA (%; median (IQR))	31.5 (21)	29 (24)	25 (18)	34 (15)	48 (28)	0.048*
BMI (kg/m^2; mean ± SD)	27.3 ± 6.1	29.4 ± 7.7	25.7 ± 4.1	26.4 ± 5.2	26.1 ± 4.2	0.057
Trauma mechanism (n, %*)						
Burn	72 (80.0%)	24 (33.3%)	10 (13.9%)	32 (44.4%)	6 (8.3%)	0.149
Scald	14 (15.6%)	7 (50.0%)	3 (21.4%)	3 (21.4%)	1 (7.1%)	
Electrical	4 (4.4%)	0	0	4 (100%)	0	
Inhalation injury (n, %)	25 (27.8%)	7 (28.0%)	2 (8%)	11 (44.0%)	5 (20%)	0.281
Diabetes mellitus (n, %)	4 (4.4%)	0	0	3 (75%)	1 (25%)	0.135
Total length of stay (d; median (IQR))	28 (40)	16 (24)	30 (35)	51 (61)	24 (93)	<0.001**
ICU length of stay (d; median (IQR))	16 (32)	5 (13)	21 (18)	29 (40)	17 (72)	<0.001**
ABSI (median (IQR))	7.5 (4)	7 (6)	7 (2)	8 (2)	11 (5)	0.113
Baux score (median (IQR))	84 (38)	84 (49)	73.5 (39)	84 (33)	104.5 (29)	0.307
Mortality (n, %)	14 (15.6%)	10 (71.4%)	0	2 (14.3%)	2 (14.3%)	0.005**
Type of infection (n, %)						
Wound infection	12 (20.3%)	—	3 (25%)	8 (66.7%)	1 (8.3%)	—
Pneumonia	34 (57.6%)	—	4 (11.8%)	26 (76.5%)	4 (11.8%)	—
Urinary tract infection	3 (5.1%)	—	3 (100%)	0	0	—
Central line infection	6 (10.2%)	—	2 (33.3%)	4 (66.7%)	0	–
Bacteremia	4 (6.8%)	—	1 (25%)	1 (25%)	2 (50%)	–

ABSI indicates abbreviated burn severity index; BMI indicates body mass index; ICU, intensive care unit; IQR, interquartile range; SD, standard deviation; TBSA, total body surface area.

*$p < 0.05$.

**$p < 0.01$.

and sepsis at day 2, 7, 8, and 10, and WBCs merely at admission. Of note, only PSP demonstrated a significant interaction between time and presence of sepsis – signifying a significantly steeper increase in PSP levels in septic patients as opposed to those exhibiting a nonseptic course (interaction $P_{PSP} < 0.001$, $P_{PCT} = 0.135$, $P_{WBCs} = 0.166$, $P_{CRP} = 0.118$) (Fig. 1).

To further corroborate the predictive ability of PSP with regard to the status of sepsis, we employed univariate binary logistic regression with receiver operating characteristic curve (ROC) curve analysis. PSP was able to predict septic courses from day 3 to day 10 after trauma with an AUC of up to 0.89 at day 7 ($P < 0.001$), thus competing with the predictive ability of PCT achieving a maximum AUC of 0.86 ($P < 0.001$) at day 8. Combination of PSP and PCT demonstrated even higher AUCs ranging between 0.90 and 0.92 at day 6–10 (Fig. 2). Only day 3 after trauma demonstrated a remarkable increase by 11% when combining the 2 markers. Combining PSP and ABSI score in the binary logistic regression model yielded no remarkable increase of accuracy (S3). CRP and WBCs showed less predictive ability with AUC below 0.8. Giving equal weight to both sensitivity and specificity, we selected 60.12 ng/mL for PSP as

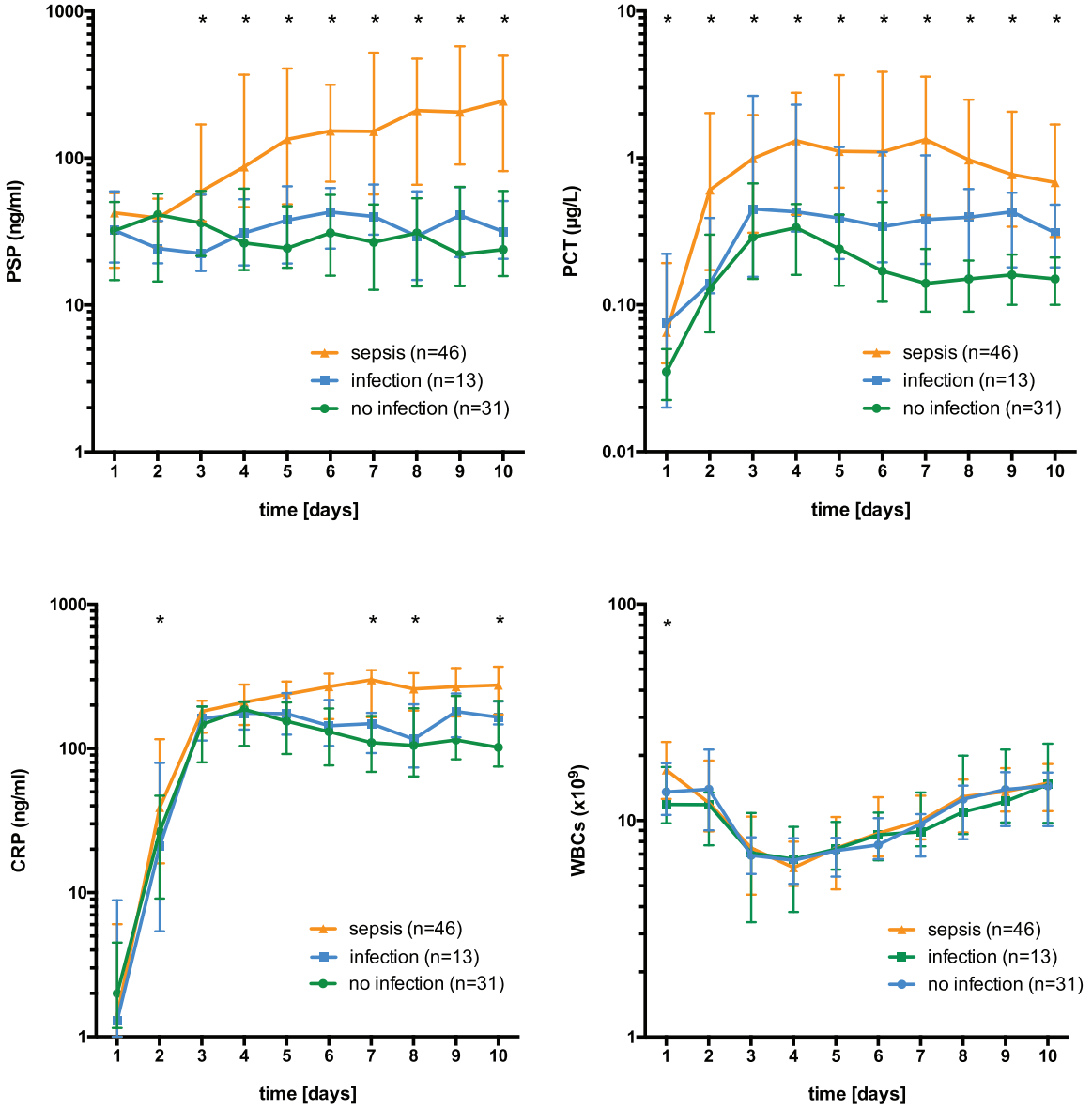

FIGURE 1. Main effects analysis determined that PSP differentiated between infection, sepsis, and sterile inflammation from day 3 ongoing, whereas PCT distinguished between the 3 groups at every time point. CRP was able to differentiate between noninfection, infection, and sepsis at day 2, 7, 8, and 10, and WBCs at admission only. PSP demonstrated a significant interaction between time and presence of sepsis – signifying a significantly steeper increase in PSP levels in septic patients as opposed to those exhibiting a nonseptic course (interaction $P_{PSP} < 0.001$, $P_{PCT} = 0.135$, $P_{WBCs} = 0.166$, $P_{CRP} = 0.118$). CRP indicates C-reactive protein; PCT, procalcitonin; PSP, pancreatic stone protein; WBCs, white blood cells.

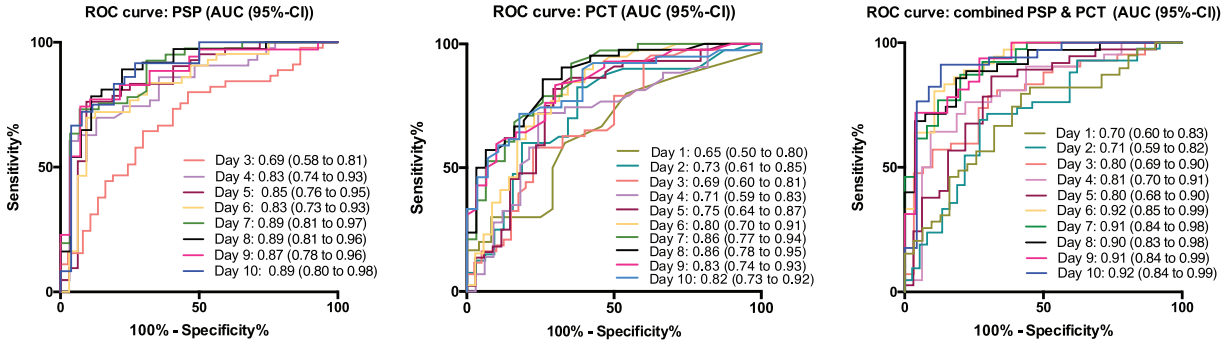

FIGURE 2. Left: Binary ROC-curve analysis with AUC and 95%-CI demonstrating PSP to predict septic courses from day 3 to day 10 after trauma with AUC of up to 0.89 at day 7. Middle: PCT showed predictive ability from admission with a maximum AUC of 0.86 at day 7. CRP and WBCs (not shown) demonstrated less predictive ability with AUC below 0.8. Right: ROC curve of combined PSP and PCT demonstrating AUCs ranging between 0.90 and 0.92. AUC indicates area under the curve; CRP, C-reactive protein; PCT, procalcitonin; PSP, pancreatic stone protein; ROC, receiver operating characteristic curve; WBCs, white blood cells.

cut-off value being able to predict yet clinically unapparent septic courses at day 7 after trauma (sensitivity/specificity: 81%, positive likelihood ratio: 4.34). PSP demonstrated no predictive power for local infections without septic complications (AUC $\approx$ 0.60, $P >$ 0.20), whereas PCT was able to predict local infections with AUC ranging between 0.70 and 0.80 ($P < 0.05$).

Event-related Analysis of Inflammatory Biomarkers

To further elucidate the interaction between time and status of disease (infection, sepsis, septic shock), we rearranged the individual biomarker courses according to their time point of occurrence and used the grand median of patients with an uneventful course as reference line. This event-related analysis demonstrated PSP serum levels to triple within 72 hours and to double within 48 hours before a clinically apparent sepsis with subsequent initiation of therapy. Likewise, PSP levels appeared 10-fold higher within 48 hours before septic shock was clinically overt. In contrast, PSP serum values hardly varied in patients with a local infection before the event (Fig. 3). PCT serum levels nearly tripled in patients with septic shock and doubled in patients with local infections within 72 hours before the event, whereas septic patients demonstrated merely marginal alterations. CRP serum levels showed an circa 1.5-fold increase in patients with local infections and septic complications within 72 hours before clinically overt. In the same time frame, CRP doubled in patients with septic shock. Eventually, serum levels of WBCs demonstrated unspecific undulations within 72 hours before diagnosis of the corresponding event.

DISCUSSION

The present study investigated the time course of PSP and 3 established pro-inflammatory biomarkers (PCT, CRP, WBC) in a cohort of 90 severely burned patients with regard to the incidence of infection and septic complications within the initial 14 days after trauma resulting in >1000 measurements per biomarker. Moreover, PSP was the only biomarker demonstrating a significant interaction between time and presence of sepsis – indicated by a significantly steeper increase in PSP levels in septic patients as opposed to those without sepsis. This interaction suggests that – besides a cut-off value – the time-related kinetics of PSP has a crucial role in the preclinical identification of septic patients. Interestingly, patients with a local infection demonstrated nearly the same values as those with no infection, suggesting for PSP to have a critical role in the sepsis pathway compared to inflammatory cascades reigning local infections or wound related inflammation. This finding reflects the high specificity of PSP with regard to septic courses and confirms the results of previous studies.[20,25]

Day-wise main effect analysis showed that PCT was able to differentiate between no infection, infection, and sepsis at all of the time points, whereas PSP demonstrated discriminatory power from day 3. On the one hand, the high performance of PCT underlines the validity of our study results because several previous studies have demonstrated PCT to be raised in burned patients with infectious and septic courses.[36–38] On the other hand, it is unlikely that an increased level of PCT at admission really signifies a yet clinically unapparent infection. In that way, PSP demonstrated a more comprehensible course with indifferent levels during the first 2 days followed by a significant increase 3 days after trauma. Corresponding binary logistic regression with ROC-curve analysis replicated the day-wise main effect analysis of the linear mixed model with PSP and PCT being highly predictive for septic events.

When combining PSP and PCT, the ROC-curve analysis achieved even higher AUCs ranging between 0.90 and 0.92 at day 6–10. On the one hand, the increase of accuracy is favorable, on the other hand, it is limited to 2%–3%. Only day 3 after trauma demonstrated a remarkable increase by 11% when combining the 2 markers. Moreover, adding the ABSI score to PSP in the binary logistic regression model yielded marginally higher AUC values at single days. One reason for this observation could be, that the ABSI score is a static score that is evaluated once and does not change over time, and, therefore, may not necessarily reflect the dynamic changes that lead to sepsis development. These data suggest, that combining PSP and PCT might be advantageous especially in the early phase after trauma, whereas the ABSI score has no further gain in sepsis identification. But it is to be noted, that standard ROC curve analysis represents a theoretical model, which does not account for time dependency of an event. This limitation of considering an event (ie, sepsis) status for an individual as fixed over time is often encountered in biomarker studies and demands cautious data interpretation. Therefore, the event-related analysis as shown in Fig. 3 compensates for that and reflects the predictive strength of a biomarker better. Against this background, PSP alone has excellent predictive ability towards septic progression, which can hardly be amplified by adding further powerful markers, such as PCT. In the authors' view and as demonstrated in previous studies, PCT rather has its strength in guiding antibiotic therapy than in predicting sepsis.[13]

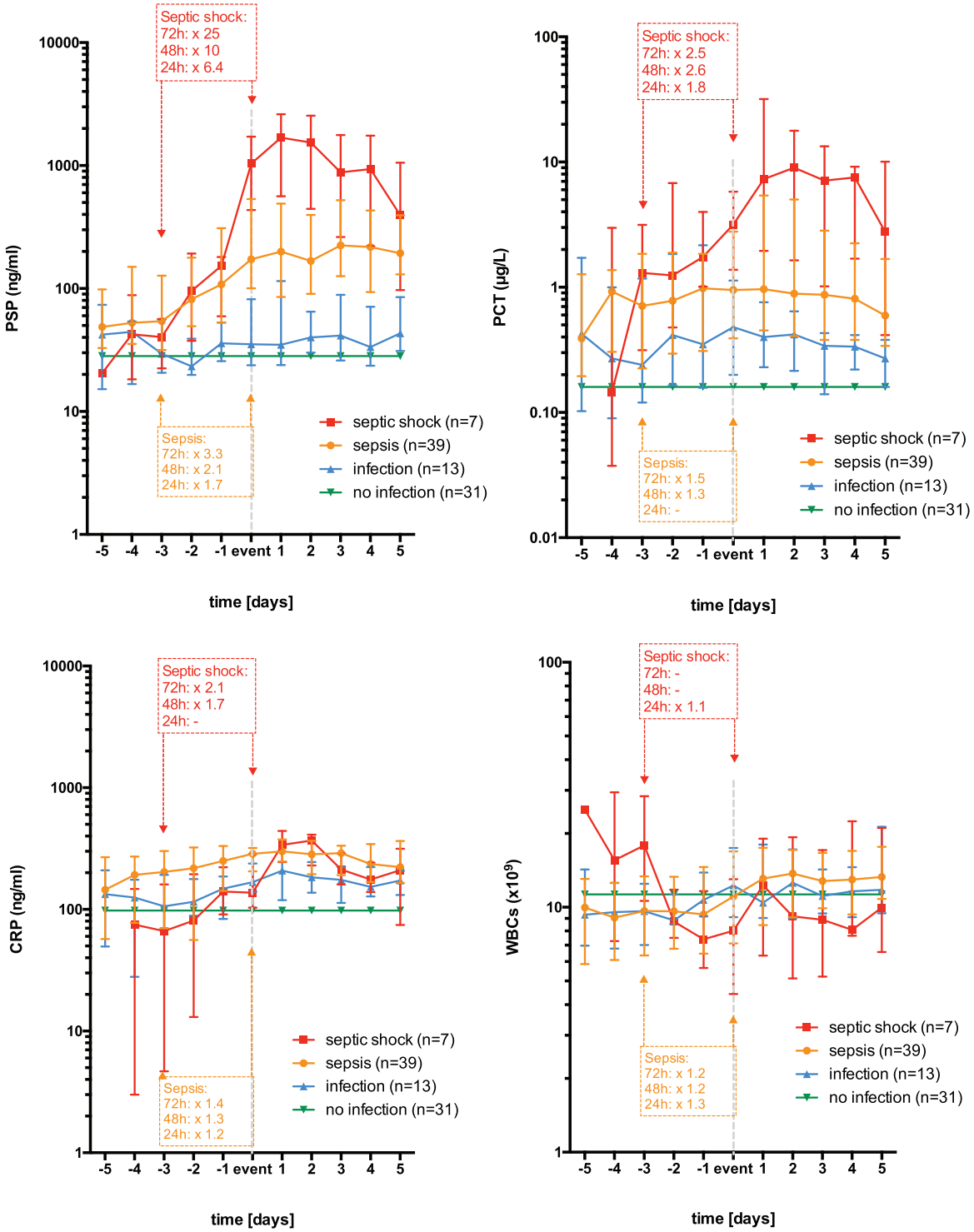

FIGURE 3. Event-related depiction of PSP and routine inflammatory biomarkers. PSP serum levels tripled within 72 h and doubled within 48 h before a clinically apparent sepsis. In septic shock, PSP levels appeared 10-fold higher within 48 h before clinically overt. PCT serum levels nearly tripled in patients with septic shock and doubled in patients with local infections within 72 h before the event, whereas septic patients demonstrated merely marginal alterations. CRP and WBC serum levels demonstrated unspecific undulations within 72 h before diagnosis of the corresponding event. The boxes in orange and red contain the relative biomarker increase for sepsis and septic shock for 72 h, 48 h, and 24 h, respectively. Note the log-transformed ordinate with different scales. CRP, C-reactive protein; PCT, procalcitonin; PSP, pancreatic stone protein; WBCs, white blood cells.

The ultimate goal in sepsis biomarker research, however, is the development of a score that is useful for sepsis diagnosis and risk stratification. With the availability of systems biology approaches, such a sepsis score may ideally comprise a combination of several biomarkers, including proteomic, transcriptomic, and metabolomic candidates as wells as clinical parameters. Examples of preliminary scores in different sepsis cohorts include the combination of clinical and transcriptomic markers,[39,40] metabolic and protein-mediator profiling,[41] or the combination of biomarkers with established clinical scores.[42] In our opinion, PSP is a very promising candidate to be included in a sepsis score in general and in particular for discrimination of septic complications in patients with underlying systemic inflammation as indicated by the results of the present study. Still, based on the experience of previous studies, the development of a multi-biomarker score for predicting and diagnosing sepsis represents an intricate endeavor.[43] The implementation of novel fields, such as bioinformatics, computational biology, and machine learning, into sepsis research might open up new possibilities to adequately reflect the complexity and dynamics of sepsis for development of a valid and reliable diagnostic tool in the future. Even if the development of a sepsis score is beyond the scope of the present manuscript, current and future efforts by our research group head towards this direction.

We identified 45.67 ng/mL as cut-off value to predict clinically unapparent sepsis at day 3 after trauma, which closely corresponds with the cut-off level of 48.1 ng/mL for cardiac surgery patients in a previous report.[21] Nevertheless, these results have to be interpreted with caution as the time-dependency of the infectious/septic event is not included in standard ROC curve analysis. The limitation of considering an event (disease) status for an individual as fixed over time is often encountered in biomarker studies, harshly restricting the external validity of the results. Therefore, we rearranged the individual course with regard to the time point of the event to account for time-dependency. In this event-related analysis, PSP outperformed the canonical biomarkers by its relative increase even 72 hours before clinical diagnosis of sepsis. Despite the small number of patients with septic shock, we realized the relevance of a steep slope of PSP before becoming clinically apparent. Even PCT did neither increase earlier in relation to the event nor was its relative rise as steep as the corresponding PSP levels. The importance of the time profile of a biomarker might even be more helpful to physicians than a cut-off value per se and has been discussed for burn patients before.[44] Accordingly, these considerations suggest that PSP kinetics help identify patients with evolving sepsis. This may result in early microbiological sampling, initiation of preemptive antibiotic therapy and prevention of fatal sepsis development with reduced costs.

Baseline PSP levels were not correlated with the relevant patient- and trauma-related characteristics (sex, age, BMI, TBSA), which is desirable for a sepsis biomarker demanding high specificity. However, the employed mixed model analysis demonstrated a significant interaction between time and TBSA over 14 days for PSP, PCT, and CRP, which is why we adjusted all subsequent analyses for TBSA. Trauma severity seems to have an influence on the serum levels of PSP (and other pro-inflammatory biomarkers), which has been described before.[20,21] But as our further analysis revealed, TBSA as surrogate parameter for trauma severity most likely functions as moderator variable because patients with higher TBSA are more susceptible to septic events expressed by higher biomarker levels than those with less TBSA.

The molecular mechanism by which PSP reacts to infection is still unclear. Graf et al and Reding et al showed that the pancreas as the main locus of synthesis senses remote organ damage and systemic stress via cytokine storms and responds by secreting PSP, particularly when associated with septic complications.[26,31] Hence, the pancreas is also considered an acute phase organ. Once released in the bloodstream, PSP plays a role in activating neutrophils.[20] Of note, PSP has been demonstrated to bear a high degree of structural homology with lectins, which are calcium-dependent glycan-binding proteins known to have a diverse range of functions, including adhesion and signaling receptors in homeostasis and innate immunity, and are crucial in the inflammatory response and leukocyte and platelet trafficking.[19]

As early decision making with the initiation of therapy is key to survival, especially in burns, inflammatory biomarkers are urgently needed aiming at preclinical detection of deterioration. Vice versa, this patient population has some major methodological advantages when it comes to testing novel biomarkers as compared to other ICU patients making up for some key limitations of previous studies: (A) Prophylactic administration of antibiotics, which is generally considered a major incommoding confounder in sepsis-related biomarker research, is strongly avoided in thermally injured patients due to resistance breeding.[20,21] (B) The burn victim itself is mostly young to middle-aged with no or few comorbidities thus harshly diminishing confounding effects. (C) ICU stay of burn victims is usually prolonged. Therefore, detailed monitoring and documentation of clinical and laboratory parameters are valuable prerequisites to retrospectively identify rapid changes in patient's condition. (D) Burn victims are thought to be more susceptible to infectious complications than any other patient population allowing for statistically balanced juxtaposition of septic versus nonseptic patients.[45] (E) Except for the minor subgroup of high voltage injuries, burns represent a rather homogenous entity with directly measurable trauma severity (ie, TBSA, degree of burn injury), which can be simply adjusted for in statistical analyses.

Elucidating the role of PSP in severely burned patients with respect to the occurrence of sepsis, the present study represents a crucial step on the way to test a potentially helpful biomarker for early sepsis detection. Besides its high predictive power and its early temporal increase, PSP can readily be measured bedside from a drop of whole blood using a nanofluid based assay (abioSCOPE, Abionic SA, Epalinges, Switzerland). This device allows quantifying PSP levels at a picomolar range within 2–5 minutes and without preanalytical work. In this context, the present study needs to be considered as hypothesis generating awaiting confirmation in future clinical trials, since yet, there are some limitations: Given the single-center design of our study, there is no external validation of our data, which has to be addressed in further studies. Likewise, measurement of biomarkers and assessment of clinical parameters were performed only once per 24 hours, neglecting potential alterations between these intervals. Moreover, the influence of the type of infection and of repetitive surgical tissue damage on PSP serum levels need to be clarified in further studies.

CONCLUSIONS

PSP demonstrates high discriminatory ability to timely identify evolving sepsis and septic shock in patients with acute severe burns. Its steep increase allows sepsis detection up to 72 hours before clinically overt deterioration, thus outperforming CRP- and PCT-based protocols for sepsis diagnosis. Affordable PSP bedside measurement might enable earlier treatment of sepsis with reduced morbidity, mortality and reduce costs for burn patients in the future.

ACKNOWLEDGMENT

The authors thank Michelle McLuckie as native speaking professional language editor for her dedicated editing of the manuscript.

REFERENCES

1. Angus DC, van der Poll T. Severe sepsis and septic shock. *N Engl J Med*. 2013;369:2063.
2. Fleischmann C, Scherag A, Adhikari NK, et al. Assessment of global incidence and mortality of hospital-treated sepsis. current estimates and limitations. *Am J Respir Crit Care Med*. 2016;193:259–272.
3. Vincent JL, Marshall JC, Namendys-Silva SA, et al. Assessment of the worldwide burden of critical illness: the intensive care over nations (ICON) audit. *Lancet Respir Med*. 2014;2:380–386.
4. Jeschke MG, Patsouris D, Stanojcic M, et al. Pathophysiologic response to burns in the elderly. *EBioMedicine*. 2015;2:1536–1548.
5. Belba MK, Petrela EY, Belba AG. Epidemiology and outcome analysis of sepsis and organ dysfunction/failure after burns. *Burns*. 2017;43:1335–1347.
6. Peck MD. Epidemiology of burns throughout the world. Part I: distribution and risk factors. *Burns*. 2011;37:1087–1100.
7. Church D, Elsayed S, Reid O, et al. Burn wound infections. *Clin Microbiol Rev*. 2006;19:403–434.
8. Greenhalgh DG. Sepsis in the burn patient: a different problem than sepsis in the general population. *Burns Trauma*. 2017;5:23.
9. Pierrakos C, Vincent JL. Sepsis biomarkers: a review. *Crit Care*. 2010;14:R15.
10. Jeschke MG, Gauglitz GG, Kulp GA, et al. Long-term persistance of the pathophysiologic response to severe burn injury. *PLoS One*. 2011;6:e21245.
11. Williams FN, Herndon DN, Jeschke MG. The hypermetabolic response to burn injury and interventions to modify this response. *Clin Plast Surg*. 2009;36:583–596.
12. Jeschke MG, Finnerty CC, Kulp GA, et al. Can we use C-reactive protein levels to predict severe infection or sepsis in severely burned patients? *Int J Burns Trauma*. 2013;3:137–143.
13. Kopterides P, Siempos II, Tsangaris I, et al. Procalcitonin-guided algorithms of antibiotic therapy in the intensive care unit: a systematic review and meta-analysis of randomized controlled trials. *Crit Care Med*. 2010;38:2229–2241.
14. van Dissel JT, van Langevelde P, Westendorp RG, et al. Anti-inflammatory cytokine profile and mortality in febrile patients. *Lancet*. 1998;351:950–953.
15. Livaditi O, Kotanidou A, Psarra A, et al. Neutrophil CD64 expression and serum IL-8: sensitive early markers of severity and outcome in sepsis. *Cytokine*. 2006;36:283–290.
16. Yan S, Tsurumi A, Que YA, et al. Prediction of multiple infections after severe burn trauma: a prospective cohort study. *Ann Surg*. 2015;261:781–792.
17. Stanojcic M, Chen P, Harrison RA, et al. Leukocyte infiltration and activation of the NLRP3 inflammasome in white adipose tissue following thermal injury. *Crit Care Med*. 2014;42:1357–1364.
18. Liu X, Ren H, Peng D. Sepsis biomarkers: an omics perspective. *Front Med*. 2014;8:58–67.
19. Eggimann P, Que YA, Rebeaud F. Measurement of pancreatic stone protein in the identification and management of sepsis. *Biomark Med*. 2019;13:135–145.
20. Keel M, Harter L, Reding T, et al. Pancreatic stone protein is highly increased during posttraumatic sepsis and activates neutrophil granulocytes. *Crit Care Med*. 2009;37:1642–1648.
21. Klein HJ, Csordas A, Falk V, et al. Pancreatic stone protein predicts postoperative infection in cardiac surgery patients irrespective of cardiopulmonary bypass or surgical technique. *PLoS One*. 2015;10:e0120276.
22. Garcia de Guadiana-Romualdo L, Berger M, Jimenez-Santos E, et al. Pancreatic stone protein and soluble CD25 for infection and sepsis in an emergency department. *Eur J Clin Invest*. 2017;47:297–304.
23. Schlapbach LJ, Graf R, Woerner A, et al. Pancreatic stone protein as a novel marker for neonatal sepsis. *Intensive Care Med*. 2013;39:754–763.
24. Que YA, Delodder F, Guessous I, et al. Pancreatic stone protein as an early biomarker predicting mortality in a prospective cohort of patients with sepsis requiring ICU management. *Crit Care*. 2012;16:R114.
25. Llewelyn MJ, Berger M, Gregory M, et al. Sepsis biomarkers in unselected patients on admission to intensive or high-dependency care. *Crit Care*. 2013;17:R60.
26. Reding T, Palmiere C, Pazhepurackel C, et al. The pancreas responds to remote damage and systemic stress by secretion of the pancreatic secretory proteins PSP/regI and PAP/regIII. *Oncotarget*. 2017;8:30162–30174.
27. Keim V, Iovanna JL, Dagorn JC. The acute phase reaction of the exocrine pancreas. Gene expression and synthesis of pancreatitis-associated proteins. *Digestion*. 1994;55:65–72.
28. Faul F, Erdfelder E, Buchner A, et al. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods*. 2009;41:1149–1160.
29. Committee IPG, Steering S, Advisory S. ISBI practice guidelines for burn care. *Burns*. 2016;42:953–1021.
30. Bimmler D, Schiesser M, Perren A, et al. Coordinate regulation of PSP/reg and PAP isoforms as a family of secretory stress proteins in an animal model of chronic pancreatitis. *J Surg Res*. 2004;118:122–135.
31. Graf R, Schiesser M, Lussi A, et al. Coordinate regulation of secretory stress proteins (PSP/reg, PAP I, PAP II, and PAP III) in the rat exocrine pancreas during experimental acute pancreatitis. *J Surg Res*. 2002;105:136–144.
32. Tobiasen J, Hiebert JM, Edlich RF. The abbreviated burn severity index. *Ann Emerg Med*. 1982;11:260–262.
33. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health careassociated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control*. 2008;36:309–332.
34. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA*. 2016;315:801–810.
35. Shek DTL, Ma CMS. Longitudinal data analyses using linear mixed models in SPSS: concepts, procedures and illustrations. *Sci World J*. 2011;11:42–76.
36. Cabral L, Afreixo V, Santos F, et al. Procalcitonin for the early diagnosis of sepsis in burn patients: a retrospective study. *Burns*. 2017;43:1427–1434.
37. Barati M, Alinejad F, Bahar MA, et al. Comparison of WBC, ESR, CRP and PCT serum levels in septic and non-septic burn cases. *Burns*. 2008;34:770–774.
38. Lavrentieva A, Papadopoulou S, Kioumis J, et al. PCT as a diagnostic and prognostic tool in burn patients. Whether time course has a role in monitoring sepsis treatment. *Burns*. 2012;38:356–363.
39. Rittirsch D, Schoenborn V, Lindig S, et al. An integrated clinico-transcriptomic approach identifies a central role of the heme degradation pathway for septic complications after trauma. *Ann Surg*. 2016;264:1125–1134.
40. Rittirsch D, Schoenborn V, Lindig S, et al. Improvement of prognostic performance in severely injured patients by integrated clinico-transcriptomics: a translational approach. *Crit Care*. 2015;19:414.
41. Mickiewicz B, Thompson GC, Blackwood J, et al. Biomarker phenotype for early diagnosis and triage of sepsis to the pediatric intensive care unit. *Sci Rep*. 2018;8:16606.
42. Shukeri W, Ralib AM, Abdulah NZ, et al. Sepsis mortality score for the prediction of mortality in septic patients. *J Crit Care*. 2018;43:163–168.
43. Reinhart K, Bauer M, Riedemann NC, et al. New approaches to sepsis: molecular diagnostics and biomarkers. *Clin Microbiol Rev*. 2012;25:609–634.
44. Egea-Guerrero JJ, Rodriguez-Rodriguez A. Sepsis biomarkers in severe burn patients: cut-off point or time profile? *Med Intensiva*. 2016;40:595–596.
45. Chipp E, Milner CS, Blackburn AV. Sepsis in burns: a review of current practice and future therapies. *Ann Plast Surg*. 2010;65:228–236.